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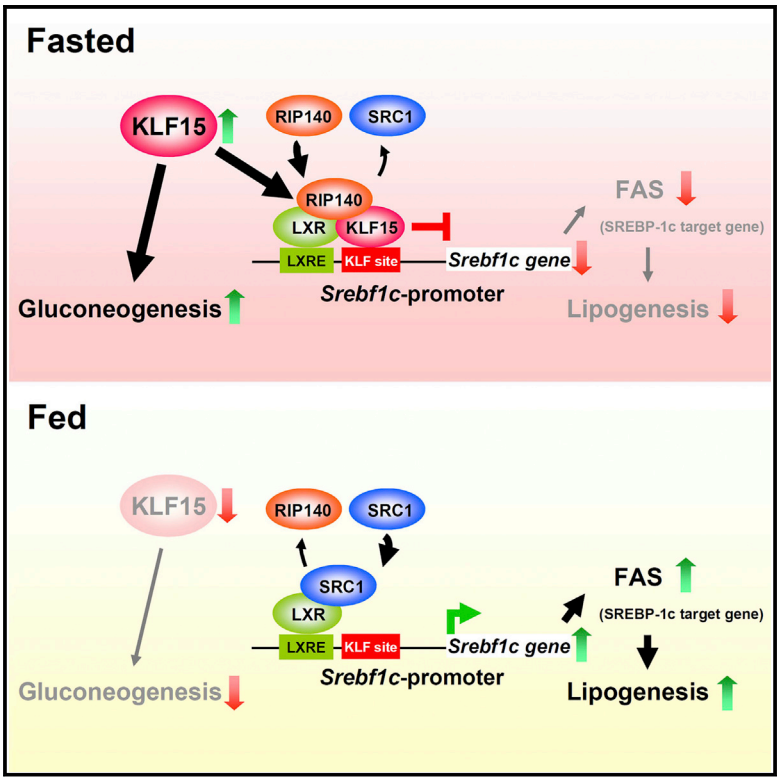
KLF15 Enables Rapid Switching between Lipogenesis and Gluconeogenesis during Fasting

Graphical Abstract



Highlights

- A transcription factor library screen reveals a complex between KLF15 and LXR/RXR
- KLF15 interacts with LXR/RXR and recruits the RIP140 corepressor to the *Srebf1c* promoter
- This enables rapid switching between lipogenesis and gluconeogenesis during fasting
- Hepatic KLF15 augmentation improves the plasma lipid profile

Authors

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In Brief

Takeuchi et al. find that KLF15 suppresses SREBP-1c transcription through interaction with the LXR/RXR/RIP140 complex. Through this mechanism, KLF15 promotes a fasting adaptation by switching lipogenesis to gluconeogenesis. KLF15 overexpression ameliorates hypertriglyceridemia without affecting LXR-mediated cholesterol metabolism.

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KLF15 Enables Rapid Switching between Lipogenesis and Gluconeogenesis during Fasting

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SUMMARY

Hepatic lipogenesis is nutritionally regulated (i.e., downregulated during fasting and upregulated during the postprandial state) as an adaptation to the nutritional environment. While alterations in the expression level of the transcription factor SREBP-1c are known to be critical for nutritionally regulated lipogenesis, upstream mechanisms governing *Srebf1* expression remain unclear. Here, we show that the fasting-induced transcription factor KLF15, a key regulator of gluconeogenesis, forms a complex with LXR/RXR, specifically on the *Srebf1* promoter. This complex recruits the corepressor RIP140 instead of the coactivator SRC1, resulting in reduced *Srebf1* and thus downstream lipogenic enzyme expression during the early and euglycemic period of fasting prior to hypoglycemia and PKA activation. Through this mechanism, KLF15 overexpression specifically ameliorates hypertriglyceridemia without affecting LXR-mediated cholesterol metabolism. These findings reveal a key molecular link between glucose and lipid metabolism and have therapeutic implications for the treatment of hyperlipidemia.

INTRODUCTION

In the postprandial state, the liver takes up nutrients through the portal vein and excess carbohydrates are converted into triglycerides, whereas in a fasting state, this lipogenic pathway shuts down. Previous studies have shown that sterol regulatory element-binding protein-1c (SREBP-1c) plays an important role in controlling hepatic lipogenesis (Shimano et al., 1999; Yahagi et al., 1999, 2002), leading us to examine the mechanism of this nutritional regulation of SREBP-1c expression.

Upon fasting, mRNA expression of the *Srebf1* gene is markedly decreased, resulting in reduced amounts of SREBP-1c protein in liver nuclei, with corresponding decreases in the mRNAs for SREBP-1-activated target genes such as fatty acid synthase (gene name, *Fasn*), a rate-limiting enzyme for lipogenesis (Horton et al., 1998). Previously, we and other authors have shown that liver X receptor (LXR), a nuclear receptor family member, transcriptionally regulates the *Srebf1* gene expression (Repa et al., 2000; Yoshikawa et al., 2001). It has also been shown that insulin-dependent (Chen et al., 2004, 2007; Kim et al., 1998; Shimomura et al., 1999; Sun et al., 2016) and insulin-independent (Haas et al., 2012; Matsuzaka et al., 2004) upstream mechanisms regulate SREBP-1 levels. Overall, the precise molecular mechanism by which LXR regulates *Srebf1* gene expression under distinct nutritional conditions remains unclear.

Here, we show through a series of experiments involving a genome-wide screening for transcription factors that fasting-induced Krüppel-like factor 15 (KLF15) interacts with LXR to repress *Srebf1* gene transcription as the primary mechanism of this nutritional regulation. The Krüppel-like family of transcription factors is a subclass of Cys2/His2 zinc-finger DNA-binding proteins, and KLF15 is expressed in multiple tissues, including the liver, white and brown adipose tissue, kidney, heart, and skeletal muscle, with the strongest expression levels occurring in the liver and kidney (Gray et al., 2002). The hepatic abundance of KLF15 is induced during the fasting state, and KLF15 is known to contribute to the regulation of gluconeogenesis in the liver (Gray et al., 2007; Teshigawara et al., 2005).

RESULTS

Identification of NuRE on the *Srebf1c* Promoter by In Vivo Ad-Luc Analyses

mRNA expression of hepatic *Srebf1* gene is tightly regulated by nutritional conditions. Using in vivo reporter gene analyses with reporter-transgenic mice, we previously showed that this nutritional regulation of the *Srebf1* gene occurs at the transcriptional

level and that a 2.2-kb region of the 5′-flanking sequence on the *Srebf1c* promoter is sufficient for this regulation (Takeuchi et al., 2007). Moreover, we previously established an intra-organ assay system to directly estimate the activity of the *Srebf1c* promoter in the livers of living animals using an adenovirus encoding the *Srebf1c* promoter linked to a luciferase reporter gene (Takeuchi et al., 2010). Using this in vivo Ad-luc promoter analysis technique, we attempted to identify the nutritional regulatory *cis* element on the *Srebf1c* promoter; luciferase reporter genes driven by different lengths of the *Srebf1c* promoter (ranging from 2,200 bp to 150 bp) were adenovirally transduced into mouse liver, and transcriptional activity was assessed by measuring luciferase activity with an IVIS imaging system (Figure 1A). From this experiment, the responsible element was found to be located within −250 to −150 bp upstream of the transcription start site. This result was confirmed by experiments using another series of adenovirus constructs encoding different partial sequences of the *Srebf1c* promoter to estimate enhancer activity (Figure S1A).

Next, we investigated the −250- to −150-bp region in detail; this region contains two LXR-binding elements (LXREs) (Yoshikawa et al., 2001). Intriguingly, the two LXREs alone were not sufficient for nutritional regulation, although they exhibited a complete response to an LXR ligand (Figure 1B), suggesting that nutritional regulation is not brought about by changes in ligand concentration. Further scrutiny clarified that a *cis* element flanking the LXRE besides the two LXREs is indispensable for nutritional regulation (Figure 1C). This element, which we designated the nutritional regulatory element (NuRE), was subsequently shown to exert a suppressive effect on the *Srebf1c* promoter in a fasting state (Figures 1D and S1B). Consistent with this finding, an electrophoretic mobility shift assay (EMSA) showed a fasting-specific band (Figure 1E), suggesting that some fasting-acting transcription factor(s) in liver nuclei that binds to NuRE may be involved in the suppression of *Srebf1c* promoter activity during fasting.

Previous reports have indicated the involvement of LXR/RXR in the regulation of *Srebf1c* promoter activity (Repa et al., 2000; Yoshikawa et al., 2001), as supported by our own data demonstrating that the simultaneous knockdown of both LXRα and β markedly reduces the nutritional response of *Srebf1c* promoter activity (Figures S1C–S1E). Based on these lines of evidence and the fact that mRNA and protein expression levels as well as DNA binding of LXRα/β were not altered by dietary manipulations (Figure 2F), we hypothesized that this unknown transcription factor binding to NuRE conferred a negative regulatory effect on LXR/RXR transcriptional activity.

TFEL Genome-wide Screen of *trans*-Acting Factors for NuRE

To identify the hypothetical transcription factor(s) that binds to NuRE and represses LXR/RXR on the *Srebf1c* promoter, we screened 1,588 genome-wide transcription factor genes included in the TFEL (Transcription Factor Expression Library; N.Y. and Y.T., unpublished data), and assessed the suppressive effects of individual transcription factors against the activity of the promoter containing NuRE plus two LXREs in the presence of cotransfected LXRα/RXRα (Figures 1F, S1F, and S1G). After three rounds of screening, two transcription factors (KLF15 and KLF4) were identified as candidates (Figures 1G and S1H). KLF15 and KLF4 suppressed the promoter containing NuRE plus two LXREs in an NuRE-dependent manner, while KLF family members other than KLF15 and KLF4 did not (Figure 2A). In addition, EMSAs demonstrated that recombinant KLF15 and KLF4 proteins bind to the NuRE probe (Figures 2B, S2A, and S2B). This element includes a 5′-CCCCATTC-3′ sequence that resembles the 5′-CCCCACCC-3′ consensus motif of KLF family members (Jiang et al., 2008).

These results were further supported by an in vivo mutation analysis, which demonstrated that the mutation at the KLF-binding site decreased the response of the native *Srebf1c* promoter activity to dietary manipulations (Figures S2C and S2D). The DNA sequences of this KLF-binding site as well as the two LXREs are highly conserved among vertebrates (Figure 2C).

Fasting-Induced KLF15 Binds to NuRE

Next, we examined KLF15 and KLF4 expression levels in the liver. Several KLFs are known to be widely expressed in various organs involved in energy metabolism (Gray et al., 2007; Mori et al., 2005; Teshigawara et al., 2005; Uchida et al., 2000). The expression of KLF15 in the liver was robust and markedly increased with fasting (Figure 2D), consistent with previous reports (Takashima et al., 2010; Teshigawara et al., 2005). By contrast, hepatic KLF4 expression was a thousand-fold lower. Accordingly, the nuclear protein detected using the EMSA to bind to NuRE during fasting was identified as KLF15 based on the disappearance of the band when adding anti-KLF15 antibody (Figure 2E). Furthermore, chromatin immunoprecipitation (ChIP) assays showed that KLF15 binding to the NuRE was markedly increased in the fasting state compared with the re-fed state (Figure 2F). In contrast, the elevation of KLF4 expression to the 2-fold level of the fasting condition using adenovirus had no effects on *Srebf1c* transcription (Figures S2E–S2H). Based on these data, we excluded KLF4 as a candidate for the NuRE-binding transcription factor and focused on KLF15 in the following studies.

Role of KLF15 in *Srebf1* Regulation

To clarify the role of KLF15 in the regulation of *Srebf1* gene expression in the in vivo liver, we examined the influences of the forced expression or the knockdown of KLF15 using adenoviruses. Interestingly, forced overexpression of KLF15 in re-fed mouse livers to levels comparable to those observed in the fasted state led to complete repression of *Srebf1c* promoter activity (Figures 3A, S3A, and S3B), a decrease in mRNA and nuclear protein levels of SREBP-1 (Figures 3B and S3C), a decrease in the promoter activity and mRNA level of *Fasn* (Figures 3B and 3C), and consequently a decrease in plasma triglyceride levels (Figure 3D). The suppressive effect of KLF15 on *Fasn* mRNA levels was completely abolished by the enforced expression of SREBP-1c (Figure S3D), demonstrating that the effect of KLF15 on hepatic lipogenesis is mediated via suppression of SREBP-1c. The adenovirally transduced KLF15 also repressed *Srebf1* expression while inducing *Pck1* (the gene name for phosphoenolpyruvate carboxykinase, the rate-limiting enzyme in gluconeogenesis) in primary hepatocytes (Figures S3E and

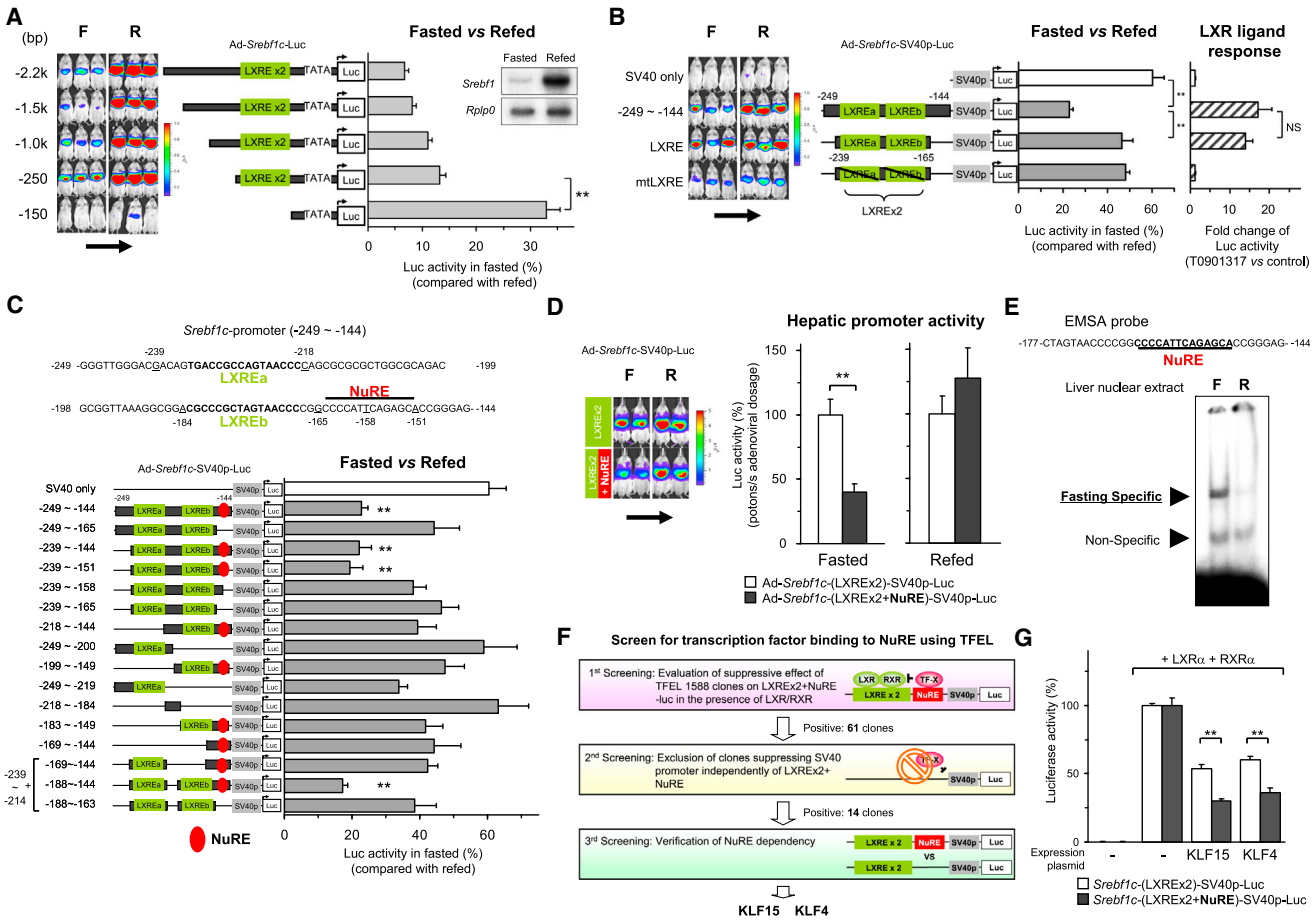

Figure 1. Identification of KLF15 through In Vivo Ad-Luc Promoter Analyses and a TFEL Genome-wide Screen

(A–D) In vivo Ad-luc promoter analyses of the *Srebf1c* gene. Hepatic luciferase activities of mice injected with Ad-*Srebf1c*-Luc (n = 5–12 per group) are shown. (A–C) Luciferase activity in the 24-hr fasted state is expressed as a percentage of that in the 16-hr re-fed state. F, fasted; R, re-fed.

(A) Various lengths of the *Srebf1c* promoter (top right: northern blot analysis of *Srebf1* expression in liver from fasted and re-fed mice).

(B) (−249 to −144) and (−239 to −165) including two LXREs and the mutated LXREs (mtLXRE) attached to an SV40 promoter. LXR ligand T0901317-administrated mice versus control mice are also shown.

(C) Various regions of the *Srebf1c* promoter attached to an SV40 promoter.

(D) Comparison between constructs with or without NuRE. Luciferase activity per transduced adenoviral DNA is shown.

(E) Electrophoretic mobility shift assay with radiolabeled probe for NuRE and liver nuclear extract proteins from fasted and re-fed mice.

(F) Processes of screening to identify transcription factors that bind to NuRE using TFEL (Transcription Factor Expression Library). In the first screening, all the 1,588 TFEL clones were cotransfected individually into HEK293 cells together with LXRα/RXRα expression plasmids and *Srebf1c*-(LXREx2+NuRE)-SV40p-Luc reporter plasmid to evaluate suppressive effects on LXREs plus NuRE. In the second screening, TFEL clones showing non-specific suppressive effects on the SV40 promoter were eliminated. In the third screening, the specificity of suppression was further verified.

(G) NuRE-dependent inhibition of KLF15 and KLF4 against *Srebf1c* promoter activity in the presence of LXRα/RXRα in HEK293 cells (n = 3 per group). Luciferase activity is expressed as a percentage of control.

Results are presented as means ± SEM. **p < 0.01 versus control of each group. See also Figure S1.

S3F). In contrast, the mRNA level of *Abca1*, another representative target gene of LXR, was not influenced by KLF15 overexpression (Figure 3B), demonstrating that this KLF15-mediated suppression of LXR is dependent on NuRE and therefore specific to the *Srebf1c* promoter.

Conversely, when we evaluated the contribution of KLF15 in the fasting state by knockdown, promoter activity as well as the expression of *Srebf1* and, consequently, *Fasn* genes was markedly elevated despite fasting conditions, whereas *Pck1* expression was significantly decreased (Figures 3E, 3F, S3G, and S3H). The two small hairpin RNAs (shRNAs) exerted essentially the same effects on expression levels of SREBP-1c, excluding the possibility of off-target effects (Figures S3I–S3K). In contrast, the mRNA level of *Abca1* was not affected by KLF15 knockdown, again showing that the KLF15-mediated inhibition of LXR is specific to the *Srebf1c* promoter. The effect of KLF15 knockdown was shown to be dependent on NuRE (Figure S3L).

To further check the validity of our model, we analyzed KLF15 knockout mice. KLF15 knockout mice exhibited significantly higher activities of *Srebf1c* promoter at an early stage of fasting

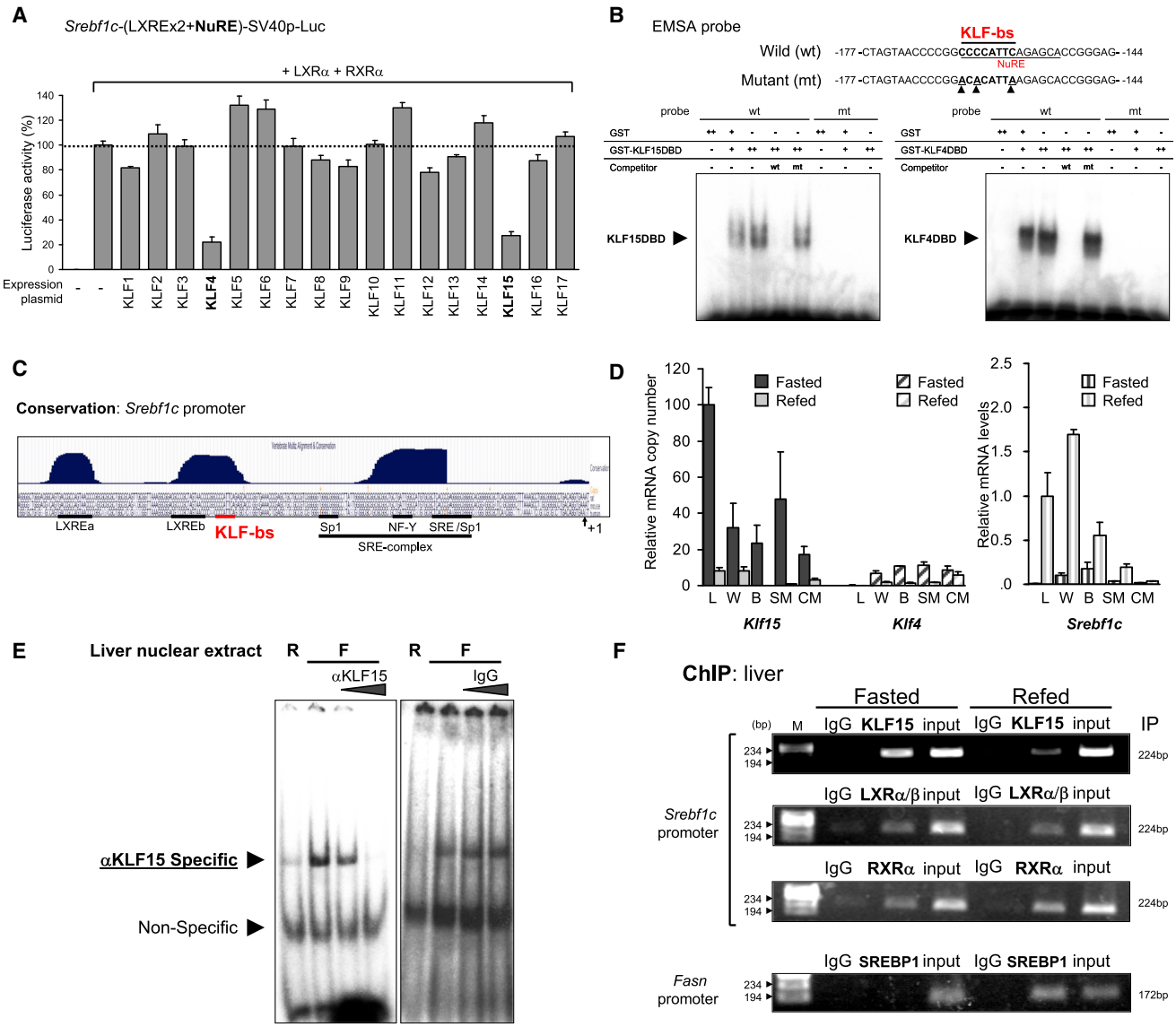

Figure 2. Fasting-Induced KLF15 Binds to NuRE on the *Srebf1c* Promoter

(A) Inhibitory effects of KLF family members on the *Srebf1c* promoter in the presence of LXRα/RXRα in HEK293 cells (n = 3 per group).

(B) Electrophoretic mobility shift assay with radiolabeled probe for NuRE and GST-KLF15DBD or GST-KLF4DBD recombinant proteins (+, 100 ng; ++, 500 ng). wt, wild-type; mt, mutant.

(C) Result of a UCSC genome browser query (http://genome.ucsc.edu/) showing conservation of the KLF-binding site in the *Srebf1c* promoter among vertebrate species.

(D) qRT PCR analysis of *Klf15* and *Klf4* mRNA copy numbers and *Srebf1c* mRNA levels in various tissues from fasted and re-fed mice (n = 3–4 per group). L, liver; W, white adipose tissue; B, brown adipose tissue; SM, skeletal muscle; CM, cardiac muscle.

(E) Electrophoretic mobility shift assay using radiolabeled probe for the KLF-binding site in the *Srebf1c* promoter and liver nuclear extracts from fasted and re-fed mice. KLF15-specific binding was shown using an anti-KLF15 antibody (immunoglobulin G [IgG] as a negative control). F, fasted; R, re-fed.

(F) Chromatin immunoprecipitation assay with liver nuclear extracts and anti-KLF15, anti-LXRα/β, anti-RXRα, and anti-SREBP1 antibodies along with IgG as a negative control. PCR was conducted with primers for two LXREs plus NuRE in the *Srebf1c* promoter or SRE in the *Fasn* promoter as a control.

Results are represented as means ± SEM.

See also Figure S2.

(6 hr of fasting) as assessed by luciferase reporter assay and quantified using in vivo imaging system (Figure 3G). In accordance with this, the rapid decrease of *Srebf1c*, *Fasn* mRNA, and plasma triglycerides (TGs) as well as the rapid increase in *Pck1* mRNA observed in wild-type mice after 6 hr of fasting was not seen in KLF15 knockout mice (Figures 3H, 3I, S4A, and S4B). These data were consistent with those from the knockdown experiments.

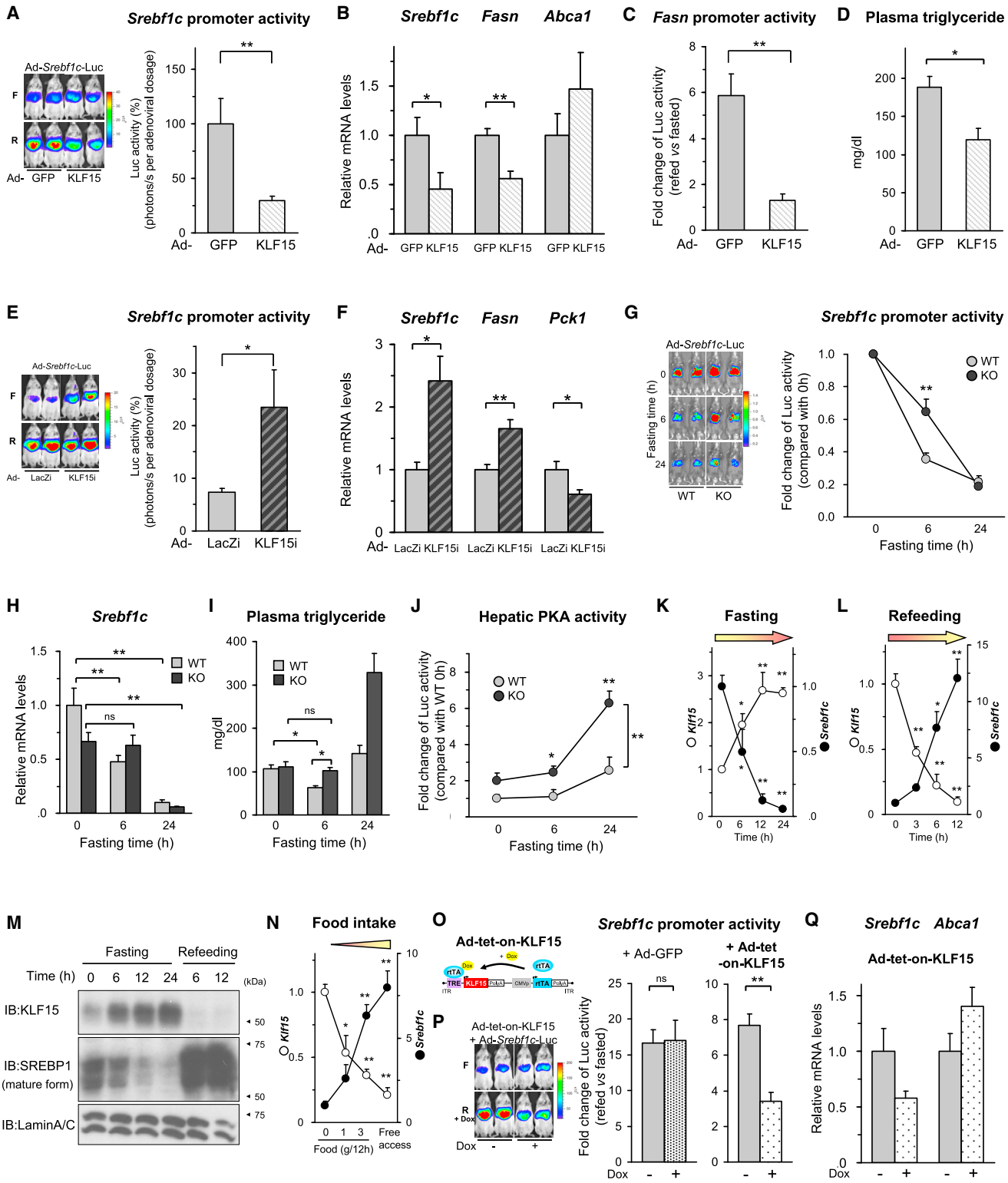

Figure 3. Role of KLF15 in *Srebf1* Regulation

(A–D) Effects of KLF15 elevation. KLF15 were elevated to the fasting level in re-fed mouse liver using an adenovirus (Ad-KLF15).

(E and F) Effects of KLF15 knockdown. Knockdown of hepatic KLF15 was performed using adenovirus-mediated RNAi (Ad-KLF15i).

(G–J) Analyses of KLF15 knockout (KO) compared with wild-type (WT) mice.

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By contrast, after prolonged fasting (24 hr of fasting), the *Srebf1c* promoter activities of KLF15 knockout mice were suppressed to the similar levels as those of the wild-type control mice. To clarify the compensatory mechanism in KLF15 knockout mice that finally suppressed SREBP-1c to the same low levels seen in wild-type mice under the prolonged (24-hr) fasting condition, we examined blood profiles of KLF15 knockout mice. Consistent with a previous report (Gray et al., 2007), KLF15 knockout mice showed significantly lower blood glucose levels (Figure S4C), leading to the marked elevation of plasma glucagon levels (Figure S4D). We found that this in turn activated the hepatic protein kinase A (PKA) pathway in KLF15 knockout mice (Figures 3J and S4E–S4G), which led to the suppression of LXR/RXR transcriptional activity (Yamamoto et al., 2007).

Consistently, the mRNA and protein abundance of *Klf15* and *Srebf1* in the liver showed reciprocal changes according to the time course of fasting or re-feeding (Figures 3K–3M). Moreover, the reverse correlation between KLF15 and SREBP-1 was preserved when animals were re-fed with various amounts of food (Figure 3N).

To further verify if this suppression by KLF15 actually occurs during the physiological time course, we checked the suppressive effect of KLF15 using the Tet-on system, where the rapid induction of KLF15 is driven by doxycycline (Dox) (Figures S4H–S4K). As shown in Figures 3O–3Q (see also Figure S4K), Dox administration quickly suppressed *Srebf1c* promoter activity.

From these multiple lines of evidence, we concluded that fasting-induced KLF15 binds to NuRE and suppresses *Srebf1c* promoter activity by inhibiting LXR/RXR and that KLF15 rapidly controls expression levels of SREBP-1c.

Mechanism of the KLF15-LXR Interaction

To elucidate the molecular mechanism of KLF15 suppression against LXR/RXR, the molecular interaction between KLF15 and LXR/RXR was assessed using coimmunoprecipitation experiments. KLF15 and LXR/RXR were shown to form a complex in cultured cell and in vivo liver nuclei (Figure 4A). Notably, the LXR-RXR interaction was not influenced by KLF15 (Figure 4B).

To understand the specificity of this KLF15-LXR/RXR interaction, we investigated the binding of KLF family members, including KLF15 and KLF4, to LXRα and RXRα using glutathione S-transferase (GST) pull-down assays. Among the KLFs that were tested (KLF1, 4, 5, 9, 10, and 15), only KLF15 and KLF4 bound to LXRα (Figure 4C). Essentially the same results were obtained using mammalian two-hybrid experiments (Figure 4D). RXRα showed a broader specificity for KLF5, KLF9, and KLF10, in addition to KLF15 and KLF4. Taken together with the finding that other KLFs such as KLF5, 9, and 10 can bind to the NuRE sequence (Figures S2A and S2B), the specificity of KLF15 and KLF4 on the suppression of *Srebf1c* promoter activity (Figure 2A) is considered to be attributable to the specific binding of LXR to KLF15 and KLF4. Intriguingly, KLF15 also has an affinity for RARα, and RAR was interchangeable with LXR as a partner for KLF15 (Figures S5A and S5B).

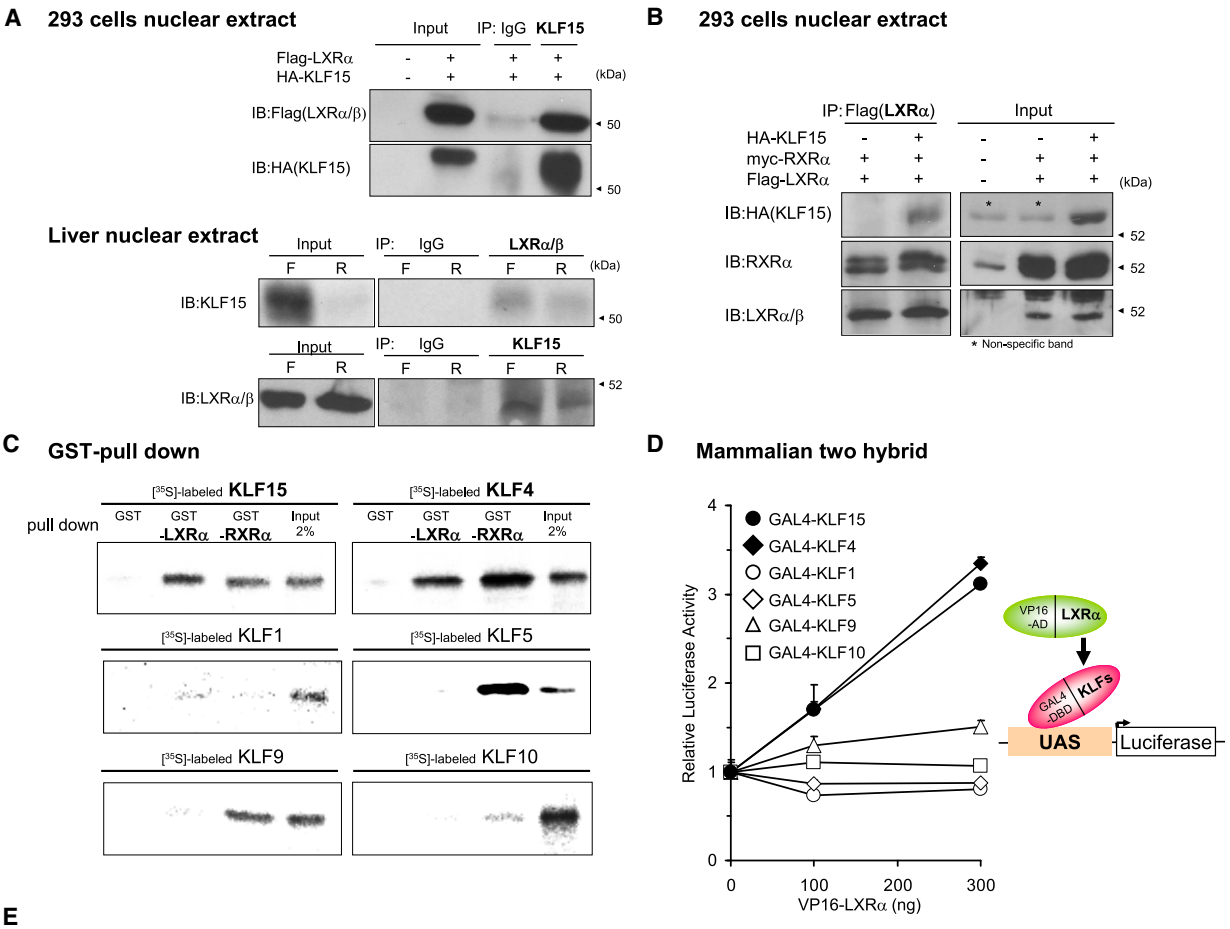
Next, we attempted to determine the domain of KLF15 responsible for the interaction with LXRα. KLF15 consists of two domains: an N-terminal repression and activation domain (RAD) and a C-terminal DNA-binding domain (DBD) composed of three zinc-finger domains. ^{35}S-labeled deletion derivatives of KLF15 were subjected to a pull-down assay with GST-LXRα, and the zinc-finger domain of KLF15 was shown to be critical for its interaction with LXRα (Figure 4E). Supporting this finding, a truncated KLF15 lacking the zinc-finger domain had no effect on the inhibition of LXR/RXR activity (Figure 4F). Similarly, to map the domain of LXRα required for this interaction, we performed pull-down assays using GST fusion proteins containing various domains of LXRα, which consists of four domains: a ligand-independent activation function domain (A/B), a DNA-binding domain (C), a ligand-binding domain (DE), and a ligand-dependent transactivation function-2 domain (AF2). These pull-down assays revealed that the C domain of LXRα is critical for its interaction with KLF15 (Figure 4G). A comparable result was observed for the RXRα-KLF15 interaction (Figure S5C).

These results demonstrate that KLF15 and LXRα interact with each other through the DNA-binding domain on each molecule. The DNA-binding domains of transcription factors, including nuclear receptors, can also function as an interacting domain with other proteins (Yin et al., 2002). The EMSA results (Figure S5D) confirmed that KLF15 and LXRα/RXRα actually form a complex on the DNA fragment containing LXRE plus NuRE, whereas the binding of KLF15 or LXRα/RXRα to each corresponding element was not affected by LXRα/RXRα or KLF15, respectively.

KLF15 Represses LXR by Recruiting the RIP140 Corepressor

To further explore the detailed mechanism of how KLF15 suppresses LXR/RXR, we attempted to identify additional cofactor(s) that may be operative. Three corepressors (NcoR1, SMRT

(A, E, and G) Changes in *Srebf1c* promoter activities. Images captured using IVIS and quantified luciferase activities per transduced adenoviral DNA in the livers of re-fed (A) or fasted (E) mice are shown (n = 7–11 per group). F, fasted; R, re-fed.
(B, F, and H) qRT PCR analysis of gene expression in the livers of re-fed (B) or fasted (F) mice from each group (n = 8 per group) and KLF15 knockout mice during fasting (H) (n = 5–12 per group).
(C) Changes in hepatic *Fasn* promoter activities (n = 7–8 per group).
(D and I) Plasma triglyceride levels of the mice injected Ad-KLF15 in the re-fed states (D), and KLF15 knockout mice during fasting (I) (n = 7–10 per group).
(J) Hepatic PKA activities in KLF15 knockout mice during fasting (n = 6–7 per group).
(K–N) Changes in hepatic *klf15* and *Srebf1* gene expression (K, L, and N) and nuclear proteins (M) in the various nutritional states. Time course during fasting (K) or re-feeding (L) and relationship with the amount of food intake (N) (n = 3 per group).
(O–Q) Quick induction of KLF15 expression in re-fed mouse liver by adenovirus using the Tet-on system (Ad-tet-on-KLF15). In the re-fed state, mice were administered 1 μg/ml doxycycline (Dox) in their drinking water for 16 hr (Ad-tet-on-KLF15, n = 7–12 per group; Ad-GFP, n = 3 per group). Schema of Ad-tet-on-KLF15 (O). Hepatic *Srebf1c* promoter activities (P). Hepatic gene expression in re-fed mice (Q). F, fasted; R, re-fed.
Results are presented as means ± SEM. *$p < 0.05$ and **$p < 0.01$ versus control of each group. See also Figures S3 and S4.



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[NcoR2], and RIP140) can reportedly be coupled with LXRα or β-mediated transcription (Glass et al., 1997; Hu et al., 2003; Jakobsson et al., 2007; Yamamoto et al., 2007). To test the possible involvement of these corepressors in the KLF15-induced inhibition of LXR activity, mammalian two-hybrid assays were performed. This system detects the binding of a VP16-AD-LXRα fusion protein and GAL4-DBD-corepressor-NID (a functional domain that interacts with nuclear receptors) in HEK293 cells (Figures 5A and S5E). Unlike NcoR1 and SMRT, the hybridization between RIP140 and LXRα was enhanced by KLF15, indicating that KLF15 promotes the recruitment of the RIP140 corepressor to LXRα. This result was confirmed by immunoprecipitation experiments (Figure 5B). In particular, the amount of LXR-RIP140 complex was much more abundant in the presence of KLF15 in wild-type mice compared with KLF15 knockout mice, although LXR and RIP140 themselves were present at the similar levels in both models of mice (Figure S5F).

We also checked the possible involvement of a major coactivator coupled with LXR, steroid receptor coactivator-1 (SRC1) (Huuskonen et al., 2004); KLF15 specifically decreased the recruitment of SRC1 to LXRα (Figure 5C), while KLF family members other than KLF15 did not (Figure S5G).

Next, we examined the physiological changes in the amount of LXR-RIP140 and LXR-SRC1 complexes during fasting and refeeding in an in vivo setting. When liver nuclear extracts were immunoprecipitated with anti-LXRα/β antibody and coprecipitated RIP140 and SRC1 were visualized using western blotting, a larger amount of LXR-RIP140 complex was found in the fasting state (Figure 5D). In contrast, SRC1 coupled with LXR was reciprocally decreased in the fasting state, suggesting that RIP140 recruitment assisted by KLF15 competes with SRC1 binding to LXR and thus inhibits transcriptional activity. A ChIP assay using RIP140 and SRC1 antibody also exhibited reciprocal patterns between RIP140 and SRC1 (Figure 5E). When we tested if RIP140 and SRC1 compete against each other when binding to LXRα using a GST pull-down assay, RIP140 inhibited SRC1 binding to LXRα and vice versa (Figure 5F). Knockdown of RIP140 caused a marked elevation in *Srebf1c* promoter activity and, consequently, *Srebf1* gene expression in the fasting state (Figures 5G and 5H), but not in the fed state (Figure S5H), consistent with a previous report (Berriel Diaz et al., 2008). Moreover, RIP140 deficiency completely cancelled the effect of KLF15 knockdown on *Srebf1c* expression in the fasting state (Figure S5I), indicating that the effect of KLF15 on *Srebf1c* expression is completely dependent on RIP140.

From these results, we concluded that KLF15 suppresses LXR by recruiting a RIP140 corepressor instead of an SRC1 coactivator.

KLF15 Ameliorates Hypertriglyceridemia

To explore the possible involvement of KLF15 in the pathogenesis of obesity-related metabolic disorders, we examined the role of KLF15 in the hypertriglyceridemia observed in genetically obese *ob/ob* mice. As shown in Figure 6A (and also in Figures S6A and S6B), the expression level of KLF15 was significantly lower in *ob/ob* mouse liver compared with control. Forced restoration of KLF15 levels in *ob/ob* mice to wild-type levels attenuated *Srebf1* overexpression and led to an improvement in hypertriglyceridemia (Figures 6B, 6C, and S6C–S6E) without an elevation in blood glucose levels (Figure 6D).

As another animal model of hypertriglyceridemia, we tested the effect of KLF15 activation on mice administered T0901317, a synthetic LXR ligand. Administration of T0901317 induced hepatic SREBP-1c and caused hypertriglyceridemia. When Ad-KLF15 adenovirus was transduced in the livers of T0901317-treated mice, *Srebf1* mRNA expression was completely repressed to vehicle-treated control levels (Figures 6E, S6F, and S6G), resulting in a decrease in *Fasn* mRNA and a concomitant decrease in plasma triglyceride levels with no effect on high-density lipoprotein (HDL) cholesterol levels (Figures 6F–6H). These results suggest that KLF15 is involved in the pathogenesis of obesity-related dyslipidemia and can be a therapeutic target for treatment.

DISCUSSION

SREBP-1c is regulated at the transcription level depending upon nutritional states, especially during cycles of fasting and refeeding. Although the *Srebf1c* promoter is controlled by LXR, the precise molecular mechanism by which SREBP-1c expression is nutritionally regulated was largely unknown. The current study clearly demonstrates, using new nutrigenomic approaches, that fasting-induced KLF15 forms a complex with LXR/RXR on the *Srebf1c* promoter to repress the expression of SREBP-1c and downstream lipogenic genes during the early and euglycemic phases of fasting (Figure 7). Conversely, in the postprandial

Figure 4. Molecular Interaction between KLF15 and LXRα

(A and B) Coimmunoprecipitation of LXRα with KLF15. Nuclear extracts from HEK293 cells expressing these indicated proteins or from mouse livers in fasted and re-fed states were immunoprecipitated with anti-KLF15, anti-LXRα/β, or anti-FLAG antibody. F, fasted; R, re-fed.

(C) GST pull-down assays showing interactions between KLFs and LXRα. ^{35}S-labeled recombinant KLF15, KLF4, KLF1, KLF5, KLF9, and KLF10 proteins generated using an in vitro transcription/translation system were pulled down using GST-LXRα fusion proteins.

(D) Mammalian two-hybrid assay to examine interactions between KLFs and LXRα in HEK293 cells cotransfected with Gal4-RE-Luc and expression vectors for VP16-LXRα and GAL4-KLFs (n = 3 per group).

(E–G) Domain mapping of the interaction between KLF15 and LXRα.

(E) ^{35}S-labeled recombinant KLF15 proteins generated using an in vitro transcription/translation system were pulled down using full-length or deletion derivatives of GST-LXRα fusion proteins. RAD, repression and activation domain; ZF, zinc finger.

(F) Functional domain analysis of KLF15 necessary for the suppression of LXRα activity in cells. HEK293 cells were cotransfected with −250-*Srebf1c*-Luc plasmids, expression plasmids for Myc-tagged LXRα and RXRα, and plasmids for KLF15, KLF15RAD, or KLF15ZFs (n = 3 per group).

(G) ^{35}S-labeled recombinant full-length or deletion derivatives of KLF15 proteins generated using an in vitro transcription/translation system were pulled down using a GST-LXRα fusion protein.

Results are presented as means ± SEM. *$p < 0.05$ and **$p < 0.01$ versus control of each group. See also Figure S5.

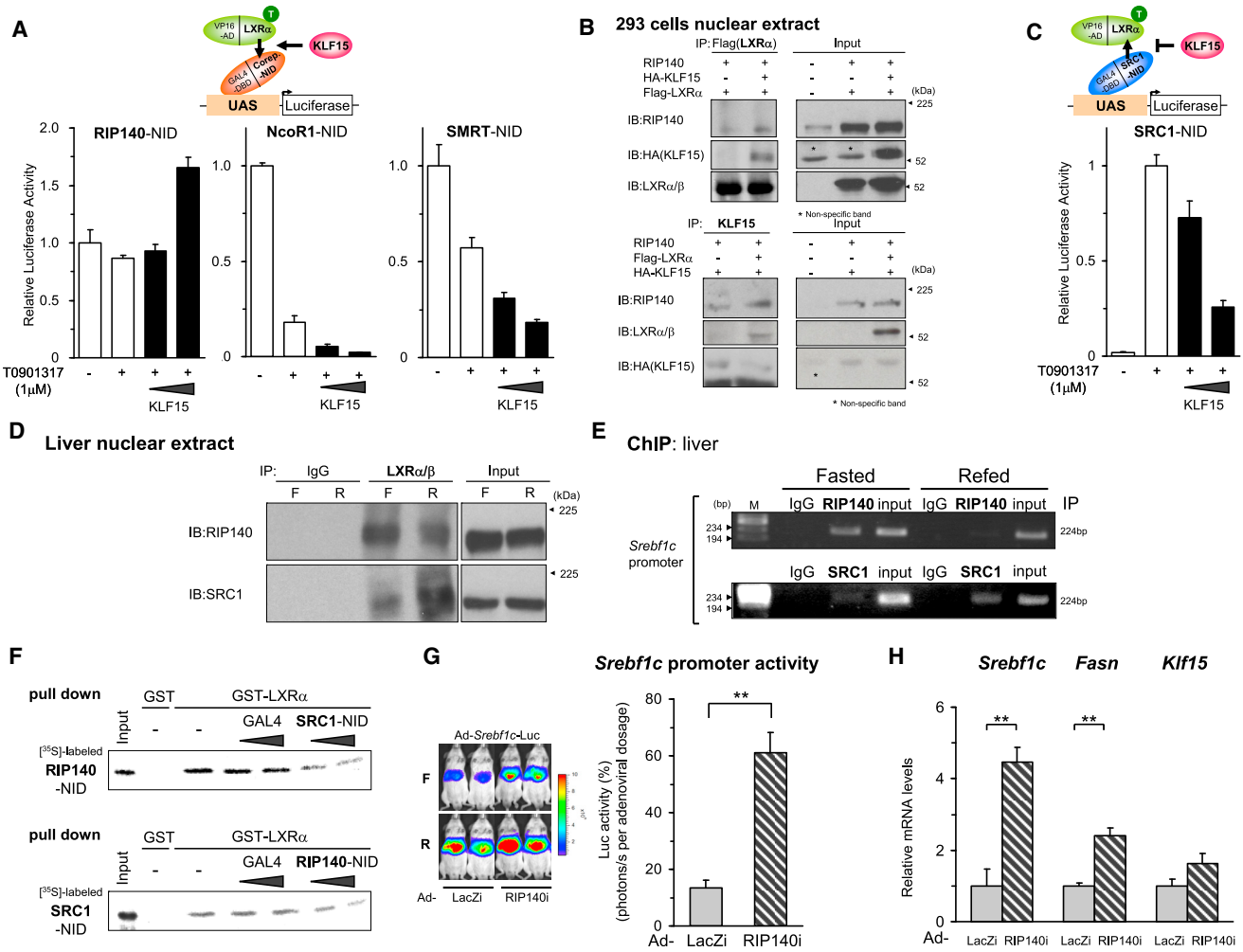

Figure 5. KLF15 Represses LXR by Recruiting the RIP140 Corepressor

(A) Recruitment of corepressors to LXRα by KLF15 as assessed using a mammalian two-hybrid system. HEK293 cells were cotransfected with Gal4-RE-Luc plasmid and expression vectors for VP16-LXRα and GAL4-Corep-NID (Gal4-DBD fused to NID of RIP140, NcoR1 or SMRT) along with KLF15 expression plasmid and incubated with media containing T0901317 (1 μM) (n = 3 per group).

(B) Coimmunoprecipitation of RIP140 with LXRα or KLF15.

(C) Recruitment of coactivator SRC1 to LXRα as assessed using a mammalian two-hybrid system (n = 3 per group).

(D) Coimmunoprecipitation of RIP140 and SRC1 using anti-LXR antibody. F, fasted; R, re-fed.

(E) Chromatin immunoprecipitation assay with anti-RIP140 and anti-SRC1 antibodies. PCR was performed using primers for two LXREs and an NuRE-containing region in the *Srebf1c* promoter.

(F) GST pull-down assay with GST-LXRα against RIP140 or SRC1. Unlabeled proteins were added as competitors.

(G and H) Knockdown of hepatic RIP140 using adenovirus-mediated RNAi (Ad-RIP140i).

(G) Images captured using IVIS and luciferase activities per transduced adenoviral DNA in the livers of fasted mice are shown (n = 5–12 per group). F, fasted; R, re-fed.

(H) qRT PCR of gene expression in the fasted mouse livers from each group (n = 7–8 per group).

Results are presented as means ± SEM. *p < 0.05 and **p < 0.01 versus control of each group. See also Figure S5.

state, KLF15 levels decrease and the suppressive complex of KLF15-LXR/RXR-RIP140 was replaced by LXR/RXR-SRC1, leading to activation of the *Srebf1c* promoter.

Before we began this study, it was well known that LXR can be activated by ligands (Forman et al., 1997; Janowski et al., 1996; Lehmann et al., 1997) and that diet-derived LXR ligands might be the key inducer of SREBP-1 and lipogenic gene expression. However, in our series of in vivo Ad-luc experiments elucidating the regulatory mechanism of LXR, we found out that the LXR-binding *cis* elements alone were not sufficient for fasting-refeeding regulation, although they exhibited a complete response to an LXR ligand (Figure 1B). This finding clearly showed that the fasting-refeeding regulation of SREBP-1c transcription is not brought about by changes in LXR ligand concentration. In accordance with this, LXR downstream genes such as ABCA1, ABCG5, and ABCG8 in the liver show no response to refeeding

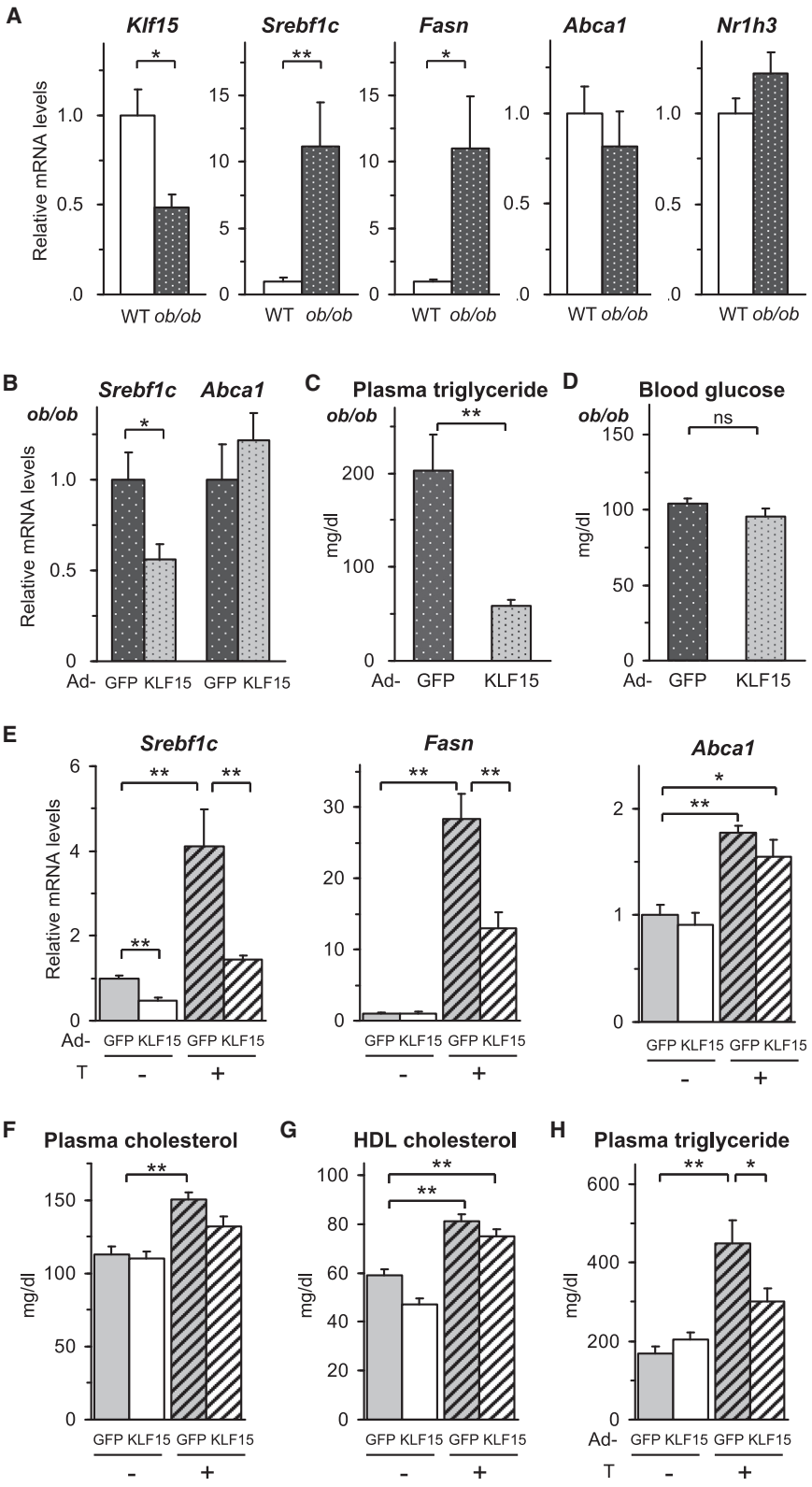

Figure 6. KLF15 Ameliorates Hypertriglyceridemia

(A) Hepatic gene expression in 18-hr-fasted *ob/ob* mice (n = 6–7 per group).

(B–D) KLF15 was elevated to wild-type levels in *ob/ob* mice liver using Ad-KLF15. Hepatic gene expression (B), plasma triglyceride levels (C), and blood glucose levels (D) in 18-hr-fasted states are shown (n = 5–6 per group).

(E–H) Effects of KLF15 activation on LXR-stimulated hyperlipidemic mice. 1 day after Ad-KLF15 injection, mice were treated orally with 50 mg/kg T0901317 once a day for 3 days in the ad lib state. (E) Hepatic gene expression at 2 hr after the last T0901317 treatment (n = 5 per group). (F–H) Plasma lipid profiles at 5 hr after the last T0901317 treatment (n = 18–25 per group).

Results are presented as means ± SEM. *$p < 0.05$ and **$p < 0.01$ versus control of each group. See also Figure S6.

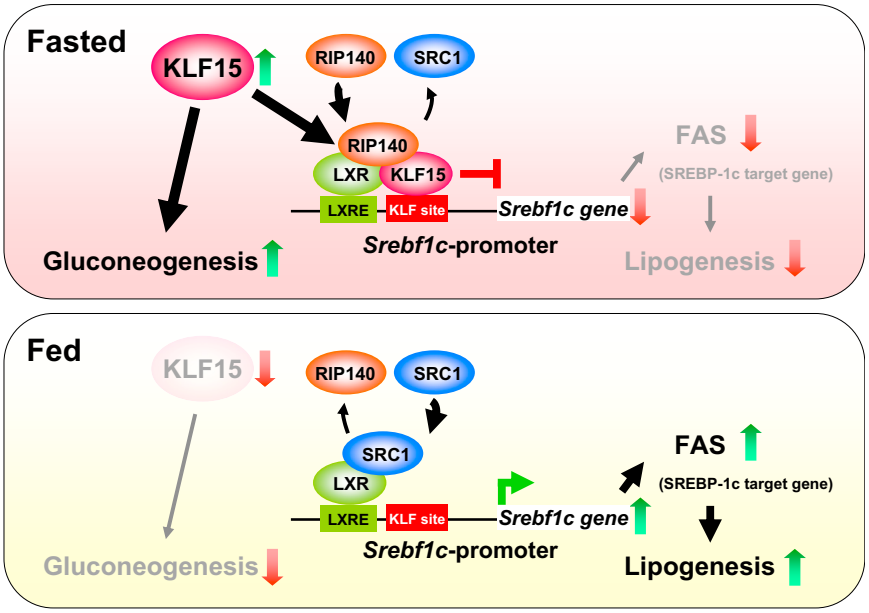

Figure 7. KLF15 Enables Rapid Switching between Lipogenesis and Gluconeogenesis
Schematic presentation of the molecular mechanism by which KLF15 switches lipogenesis and gluconeogenesis. See also Figure S7.

(Oosterveer et al., 2008). It is also notable that another nuclear receptor RAR besides LXR can function as a partner for KLF15 in this context (Figures S5A and S5B). From these lines of evidence, we concluded that LXR ligands are not involved in the fasting-refeeding response of lipogenic genes in the liver.

KLF15 is upregulated in fasting mouse liver and is known to contribute to the regulation of hepatic gluconeogenesis (Gray et al., 2007; Teshigawara et al., 2005). In light of the current work, we propose that the regulation of lipogenesis and gluconeogenesis is coordinated by KLF15 and is reciprocally regulated in response to nutritional conditions. Given that lipogenesis is an adaptive process that facilitates survival during subsequent starvation, it is conceivable that the key factor or factors controlling lipogenesis are negatively regulated by these fasting-induced factors.

In our previous publication, we reported that the PKA phosphorylation of LXR suppresses *Srebf1c* transcription by recruiting a co-repressor NcoR1 (Yamamoto et al., 2007). To clarify the relationship between KLF15-mediated and PKA-mediated *Srebf1c* repression, we analyzed KLF15 knockout mice (Gray et al., 2007). As shown in Figure 3G, KLF15 knockout mice exhibited significantly higher *Srebf1c* promoter activity at an early stage of fasting (6 hr of fasting). In contrast, after prolonged fasting (24 hr of fasting), *Srebf1c* promoter activity in KLF15 knockout mice was suppressed to levels similar to those of wild-type control mice. A series of experiments revealed that the compensatory mechanism in KLF15 knockout mice that finally suppressed *Srebf1c* expression to the same low levels seen in wild-type after prolonged fasting was hypoglycemia-induced PKA activation, which caused a suppression of LXR/RXR transcriptional activity that was dependent on the on NcoR1 co-repressor (Figures 3J, S4C–S4E, and S7A–S7E). Supporting this finding, NcoR1 binding to the *Srebf1c* promoter as assessed by ChIP assay was elevated in KLF15 knockout mice (Figure S7C). Interestingly, PKA-mediated phosphorylation of LXR did not affect RIP140 binding to LXR (Figure S5E), demonstrating that KLF15-RIP140-mediated repression is independent of PKA-NcoR1-mediated SREBP-1c repression.

This pathway from hypoglycemic PKA stimulation to LXR suppression via NcoR1 is usually activated in the prolonged fasting state, whereas KLF15-mediated lipogenic suppression is seen from the early and euglycemic periods. It is conceivable that this earlier shutdown of lipogenesis through the KLF15 pathway may help restart triglyceride synthesis after refeeding sooner in wild-type mice than in KLF15 knockout mice (Figure S7F). Together with the other important role of KLF15 in gluconeogenesis, the KLF15-mediated pathway can be considered as an adaptive and homeostatic mechanism of energy metabolism working from the euglycemic phase of fasting before hypoglycemia and PKA activation proceeds.

Previously, a detailed mutation analysis of SREBP-1c promoter activity in primary hepatocytes was reported (Chen et al., 2004), with no derepression of promoter activity around the NuRE region. Whereas our analyses were based on intra-organ assays using living animals, Chen et al. used cultured primary hepatocytes in their experiments, which might be the cause of this discrepancy. In fact, the expression of KLF15 severely diminishes soon after primary hepatocytes are prepared (data not shown), demonstrating the differences between in vivo and in vitro settings. It is probable that our strategy of starting from intra-organ assays to screen the *cis* elements enabled us to successfully identify the KLF15-mediated pathway.

Regarding the upstream regulatory mechanisms by which KLF15 is induced during fasting, the possible involvement of insulin was examined (Figure S7G). We found that STZ-induced insulin deficiency partially increased KLF15 while it decreased SREBP-1c, suggesting some molecular link between insulin and KLF15. At the same time, it has been reported that hormones other than insulin are involved in the regulation of KLF15 expression (Teshigawara et al., 2005). These findings are consistent with a previous report analyzing liver-specific insulin receptor knockout (LIRKO) mice (Haas et al., 2012), which revealed that although insulin signaling is necessary for maximal induction, nutrients (either directly or indirectly, via hormones other than insulin) are sufficient for the induction of the SREBP-1c and lipogenic gene transcripts.

In the present study, we used our original library of transcription factors, TFEL (N.Y. and Y.T, unpublished data), which

covers nearly all the known transcription factors encoded by the mouse genome, to identify the transcription factor binding to NuRE in the presence of LXR/RXR. It is well known that binding site prediction based on binding motif databases suffers from high false-positive and false-negative rates in general (Kim and Park, 2011). In particular, the sequence-based approach has no power to predict transcription factor complexes formed by protein-protein interactions.

Conversely, the search for protein-protein interactions among transcription factors alone may not be sufficient to elucidate the regulatory complexes in certain situations, including the present case, because complexes like KLF15-LXR/RXR also depend on *cis* elements (i.e., NuRE plus LXRE, in this case). This relatively weak interaction supported by the DNA backbone enables gene-specific regulations and may give more diversity to transcriptional networks. Thus, our combinatorial strategy of searching for a functional *cis* element and screening for the corresponding transcription factor complex in a context-dependent manner can be a very effective and powerful approach for exploring sophisticated transcriptional networks in detail.

Synthetic LXR ligands have been expected to have atheroprotective effects through the promotion of reverse cholesterol transport and inhibition of intestinal cholesterol absorption (Joseph et al., 2002). However, the unfavorable side effect of elevating plasma triglyceride levels has hampered the therapeutic application of LXR ligands. Our finding that KLF15 can specifically block the lipogenic effect of an LXR ligand provides therapeutic implications for the treatment of dyslipidemia, including obesity-related diseases with SREBP-1c overexpression. Moreover, it is notable that KLF15 overexpression in mouse liver, in contrast to primary cultured hepatocytes, whose expression levels of KLF15 and gluconeogenic genes are very low (data not shown), does not lead to an excess gluconeogenesis (Figures 6D, S3F, and S6D; Table S1). Thus, KLF15 overexpression can improve the plasma lipid profile without exacerbating hyperglycemia. Elucidating the upstream mechanisms of KLF15 regulation will also be important from this therapeutic point of view.

EXPERIMENTAL PROCEDURES

Animals

6- to 8-week-old ICR male mice were purchased from Central Laboratories for Experimental Animals (CLEA). 6- to 7-week-old *ob/ob* male mice were purchased from SLC. KLF15 knockout mice were a gifted from Prof. M.K. Jain and genotyped as previously described (Fisch et al., 2007), and wild-type littermates were used as controls. All animals were maintained in a temperature-controlled environment with a 12-hr light/dark cycle and given free access to standard laboratory food and water. For the fasting group, animals were starved 24 hr, and for the refeeding group, they were re-fed for 16 hr after a 24-hr starvation. For experiments using *ob/ob* mice, animals were starved for 18 hr. For LXR ligand experiments of hepatic luciferase activity, T0901317 was administered at a dose of 50 mg/kg after a 24-hr starvation and analyzed 16 hr later. For experiments using insulin-depleted diabetic mice, ICR male mice were administrated streptozotocin (two intraperitoneal injections of 100 mg/kg body weight with a 1-day interval) as previously described (Takeuchi et al., 2007). Mice were sacrificed in the early light phase in a fasted, re-fed, or nonfasted (ad libitum) state. All animals studied were anesthetized and euthanized according to a protocol approved by the Tsukuba University Animal Care and Use Committee. All experiments were repeated at least twice.

Preparation of Recombinant Adenoviruses

Recombinant adenoviruses were constructed using the Gateway system (Invitrogen). Details are provided in Supplemental Experimental Procedures.

In Vivo Imaging of Luciferase Activity

In vivo imaging was performed as described previously (Takeuchi et al., 2010). 3–6 days after adenovirus transduction, D-luciferin potassium salt (Wako) was injected intraperitoneally (i.p.) into mice, and luminescence in the liver was captured using an IVIS Imaging System (Xenogen). Relative photon emission over the liver region was quantified using LivingImage software (Xenogen). When hepatic transduction efficiency was determined, quantification of adenoviral DNA in the liver was performed using a previously described qRT-PCR method (Takeuchi et al., 2007), and the result of quantification was used to normalize the in vivo imaging of luciferase activity. Otherwise, paired data from the same animal for the different nutritional conditions (i.e., fasted or re-fed) were continuously obtained, and the ratio between the two quantities was used to cancel the variations in hepatic transduction efficiencies.

Genome-wide Transcription Factor Screening

TFEL clones (N.Y. and Y.T., unpublished data) were cotransfected with the *Srebf1c*-(LXREx2+NuRE)-SV40p-Luc plasmid together with LXRα/RXRα expression plasmids into HEK293 cells. The luciferase activity in transfectants was then measured using a luminometer with a standard assay kit (Promega).

Electrophoretic Mobility Shift Assay

EMSAs were performed as described previously (Amemiya-Kudo et al., 2000). Details are provided in Supplemental Experimental Procedures.

Chromatin Immunoprecipitation Assay

Separation of hepatic nuclei from mouse liver and ChIP assays were performed as described previously (Ide et al., 2004; Nakagawa et al., 2006). Chromatin DNA was further purified with the Wizard DNA Clean-Up System (Promega) and used as a template for PCR. Primers used to amplify the *Srebf1c* promoter or SRE (sterol regulatory element) in *Fasn* promoter regions and reaction conditions were described previously (Sekiya et al., 2007; Yamamoto et al., 2007). As a positive control, 1% of the input genomic DNA was used.

RNA Isolation and Northern Blotting

Total RNA preparation and blot hybridization with cDNA probes were performed as previously described (Takeuchi et al., 2010). Full-length cDNAs were used for cDNA probes for mouse *Klf15* and *Klf4*. The cDNA probe for mouse *Hpd* was prepared by RT-PCR using mouse liver total RNA as a template. The primers were as follows: 5′-CATTTCCACTCGGTGACCT-3′ and 5′-TGTCTTGCTCCACCCATG-3′. *Rplp0* was used as a loading control. Blots were exposed to a BAS imaging plate for the BAS2000 BIO Imaging Analyzer (Fuji Photo Film).

qRT-PCR

Total RNA (2 μg) was reverse transcribed using the High Capacity cDNA reverse transcription kit (Applied Biosystems). qRT-PCR was performed using SYBR green dye (Kapa Biosystems) on a 7300 real-time PCR system (Applied Biosystems). Primer sets are listed in Table S2.

Immunoblotting and Immunoprecipitation

Immunoblotting and immunoprecipitation were performed as described previously (Takeuchi et al., 2010; Yamamoto et al., 2007). Nuclear extract protein from mouse liver and HEK293 cells was prepared as previously described (Sheng et al., 1995; Yahagi et al., 2003, 2004).

Construction of the Tet-On System

A Tet-on system to rapidly activate KLF15 expression was constructed as described previously (Urlinger et al., 2000). A modified rTetR fragment was amplified by PCR from pUHD172-1 (Gossen et al., 1995). Tet response element (TRE) was cloned from pSIREN-RetroQ-TetP (Clontech). Details are provided in Supplemental Experimental Procedures.

GST Pull-Down Assay

GST and GST fusion proteins were prepared as described previously (Najima et al., 2005) and dialyzed with dialysis buffer (50 mM Tris-HCl [pH 8.0], 50 mM NaCl, 1 mM EDTA, and 1 mM DTT). [^{35}S]Methionine-labeled proteins were prepared using the TNT T7 quick-coupled transcription/translation system (Promega). GST pull-down assays were performed as described previously (Najima et al., 2005; Yamamoto et al., 2007).

Mammalian Two-Hybrid Assay

HEK293 cells were cotransfected with GAL4-RE-Luc plasmids and expression plasmids for GAL4 fusion protein and VP16 fusion protein.

Statistical Analyses

Data are expressed as means ± SEM. Differences between two groups were assessed using an unpaired two-tailed Student's t test. Datasets involving more than two groups were assessed by ANOVA with Statview Software (BrainPower). Differences were considered statistically significant at $p < 0.05$ ($^{*}p < 0.05$ and $^{**}p < 0.01$).

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, seven figures, and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2016.07.069.

AUTHOR CONTRIBUTIONS

N.Y. and Y.T. conceived the experiments. Y.T. performed the experiments and analyzed the data, together with N.Y. Y.L. and M.J. provided the KLF15 knockout mice. N.Y. and Y.T. co-wrote the paper. All authors discussed the results and commented on the manuscript.

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REFERENCES

Amemiya-Kudo, M., Shimano, H., Yoshikawa, T., Yahagi, N., Hasty, A.H., Okazaki, H., Tamura, Y., Shionoiri, F., Iizuka, Y., Ohashi, K., et al. (2000). Promoter analysis of the mouse sterol regulatory element-binding protein-1c gene. J. Biol. Chem. *275*, 31078–31085.

Berriel Diaz, M., Krones-Herzig, A., Metzger, D., Ziegler, A., Vegiopoulos, A., Klingenspor, M., Müller-Decker, K., and Herzig, S. (2008). Nuclear receptor cofactor receptor interacting protein 140 controls hepatic triglyceride metabolism during wasting in mice. Hepatology *48*, 782–791.

Chen, G., Liang, G., Ou, J., Goldstein, J.L., and Brown, M.S. (2004). Central role for liver X receptor in insulin-mediated activation of Srebp-1c transcription and stimulation of fatty acid synthesis in liver. Proc. Natl. Acad. Sci. USA *101*, 11245–11250.

Chen, W., Chen, G., Head, D.L., Mangelsdorf, D.J., and Russell, D.W. (2007). Enzymatic reduction of oxysterols impairs LXR signaling in cultured cells and the livers of mice. Cell Metab. *5*, 73–79.

Fisch, S., Gray, S., Heymans, S., Haldar, S.M., Wang, B., Pfister, O., Cui, L., Kumar, A., Lin, Z., Sen-Banerjee, S., et al. (2007). Kruppel-like factor 15 is a regulator of cardiomyocyte hypertrophy. Proc. Natl. Acad. Sci. USA *104*, 7074–7079.

Forman, B.M., Ruan, B., Chen, J., Schroepfer, G.J., Jr., and Evans, R.M. (1997). The orphan nuclear receptor LXRalpha is positively and negatively regulated by distinct products of mevalonate metabolism. Proc. Natl. Acad. Sci. USA *94*, 10588–10593.

Glass, C.K., Rose, D.W., and Rosenfeld, M.G. (1997). Nuclear receptor coactivators. Curr. Opin. Cell Biol. *9*, 222–232.

Gossen, M., Freundlieb, S., Bender, G., Müller, G., Hillen, W., and Bujard, H. (1995). Transcriptional activation by tetracyclines in mammalian cells. Science *268*, 1766–1769.

Gray, S., Feinberg, M.W., Hull, S., Kuo, C.T., Watanabe, M., Sen-Banerjee, S., DePina, A., Haspel, R., and Jain, M.K. (2002). The Krüppel-like factor KLF15 regulates the insulin-sensitive glucose transporter GLUT4. J. Biol. Chem. *277*, 34322–34328.

Gray, S., Wang, B., Orihuela, Y., Hong, E.G., Fisch, S., Haldar, S., Cline, G.W., Kim, J.K., Peroni, O.D., Kahn, B.B., and Jain, M.K. (2007). Regulation of gluconeogenesis by Krüppel-like factor 15. Cell Metab. *5*, 305–312.

Haas, J.T., Miao, J., Chanda, D., Wang, Y., Zhao, E., Haas, M.E., Hirschey, M., Vaitheesvaran, B., Farese, R.V., Jr., Kurland, I.J., et al. (2012). Hepatic insulin signaling is required for obesity-dependent expression of SREBP-1c mRNA but not for feeding-dependent expression. Cell Metab. *15*, 873–884.

Horton, J.D., Bashmakov, Y., Shimomura, I., and Shimano, H. (1998). Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice. Proc. Natl. Acad. Sci. USA *95*, 5987–5992.

Hu, X., Li, S., Wu, J., Xia, C., and Lala, D.S. (2003). Liver X receptors interact with corepressors to regulate gene expression. Mol. Endocrinol. *17*, 1019–1026.

Huuskonen, J., Fielding, P.E., and Fielding, C.J. (2004). Role of p160 coactivator complex in the activation of liver X receptor. Arterioscler. Thromb. Vasc. Biol. *24*, 703–708.

Ide, T., Shimano, H., Yahagi, N., Matsuzaka, T., Nakakuki, M., Yamamoto, T., Nakagawa, Y., Takahashi, A., Suzuki, H., Sone, H., et al. (2004). SREBPs suppress IRS-2-mediated insulin signalling in the liver. Nat. Cell Biol. *6*, 351–357.

Jakobsson, T., Osman, W., Gustafsson, J.A., Zilliacus, J., and Wärnmark, A. (2007). Molecular basis for repression of liver X receptor-mediated gene transcription by receptor-interacting protein 140. Biochem. J. *405*, 31–39.

Janowski, B.A., Willy, P.J., Devi, T.R., Falck, J.R., and Mangelsdorf, D.J. (1996). An oxysterol signalling pathway mediated by the nuclear receptor LXR alpha. Nature *383*, 728–731.

Jiang, J., Chan, Y.-S., Loh, Y.-H., Cai, J., Tong, G.-Q., Lim, C.-A., Robson, P., Zhong, S., and Ng, H.-H. (2008). A core Klf circuitry regulates self-renewal of embryonic stem cells. Nat. Cell Biol. *10*, 353–360.

Joseph, S.B., McKilligin, E., Pei, L., Watson, M.A., Collins, A.R., Laffitte, B.A., Chen, M., Noh, G., Goodman, J., Hagger, G.N., et al. (2002). Synthetic LXR ligand inhibits the development of atherosclerosis in mice. Proc. Natl. Acad. Sci. USA *99*, 7604–7609.

Kim, T.M., and Park, P.J. (2011). Advances in analysis of transcriptional regulatory networks. Wiley Interdiscip. Rev. Syst. Biol. Med. *3*, 21–35.

Kim, J.B., Sarraf, P., Wright, M., Yao, K.M., Mueller, E., Solanes, G., Lowell, B.B., and Spiegelman, B.M. (1998). Nutritional and insulin regulation of fatty acid synthetase and leptin gene expression through ADD1/SREBP1. J. Clin. Invest. *101*, 1–9.

Lehmann, J.M., Kliewer, S.A., Moore, L.B., Smith-Oliver, T.A., Oliver, B.B., Su, J.L., Sundseth, S.S., Winegar, D.A., Blanchard, D.E., Spencer, T.A., and Willson, T.M. (1997). Activation of the nuclear receptor LXR by oxysterols defines a new hormone response pathway. J. Biol. Chem. *272*, 3137–3140.

Matsuzaka, T., Shimano, H., Yahagi, N., Amemiya-Kudo, M., Okazaki, H., Tamura, Y., Iizuka, Y., Ohashi, K., Tomita, S., Sekiya, M., et al. (2004). Insulin-independent induction of sterol regulatory element-binding protein-1c expression in the livers of streptozotocin-treated mice. Diabetes *53*, 560–569.

Mori, T., Sakaue, H., Iguchi, H., Gomi, H., Okada, Y., Takashima, Y., Nakamura, K., Nakamura, T., Yamauchi, T., Kubota, N., et al. (2005). Role of Krüppel-like factor 15 (KLF15) in transcriptional regulation of adipogenesis. J. Biol. Chem. *280*, 12867–12875.

Najima, Y., Yahagi, N., Takeuchi, Y., Matsuzaka, T., Sekiya, M., Nakagawa, Y., Amemiya-Kudo, M., Okazaki, H., Okazaki, S., Tamura, Y., et al. (2005). High mobility group protein-B1 interacts with sterol regulatory element-binding proteins to enhance their DNA binding. J. Biol. Chem. *280*, 27523–27532.

Nakagawa, Y., Shimano, H., Yoshikawa, T., Ide, T., Tamura, M., Furusawa, M., Yamamoto, T., Inoue, N., Matsuzaka, T., Takahashi, A., et al. (2006). TFE3 transcriptionally activates hepatic IRS-2, participates in insulin signaling and ameliorates diabetes. Nat. Med. *12*, 107–113.

Oosterveer, M.H., van Dijk, T.H., Grefhorst, A., Bloks, V.W., Havinga, R., Kuipers, F., and Reijngoud, D.J. (2008). Lxralpha deficiency hampers the hepatic adaptive response to fasting in mice. J. Biol. Chem. *283*, 25437–25445.

Repa, J.J., Liang, G., Ou, J., Bashmakov, Y., Lobaccaro, J.-M.A., Shimomura, I., Shan, B., Brown, M.S., Goldstein, J.L., and Mangelsdorf, D.J. (2000). Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRalpha and LXRbeta. Genes Dev. *14*, 2819–2830.

Sekiya, M., Yahagi, N., Matsuzaka, T., Takeuchi, Y., Nakagawa, Y., Takahashi, H., Okazaki, H., Iizuka, Y., Ohashi, K., Gotoda, T., et al. (2007). SREBP-1-independent regulation of lipogenic gene expression in adipocytes. J. Lipid Res. *48*, 1581–1591.

Sheng, Z., Otani, H., Brown, M.S., and Goldstein, J.L. (1995). Independent regulation of sterol regulatory element-binding proteins 1 and 2 in hamster liver. Proc. Natl. Acad. Sci. USA *92*, 935–938.

Shimano, H., Yahagi, N., Amemiya-Kudo, M., Hasty, A.H., Osuga, J., Tamura, Y., Shionoiri, F., Iizuka, Y., Ohashi, K., Harada, K., et al. (1999). Sterol regulatory element-binding protein-1 as a key transcription factor for nutritional induction of lipogenic enzyme genes. J. Biol. Chem. *274*, 35832–35839.

Shimomura, I., Bashmakov, Y., Ikemoto, S., Horton, J.D., Brown, M.S., and Goldstein, J.L. (1999). Insulin selectively increases SREBP-1c mRNA in the livers of rats with streptozotocin-induced diabetes. Proc. Natl. Acad. Sci. USA *96*, 13656–13661.

Sun, X., Haas, M.E., Miao, J., Mehta, A., Graham, M.J., Crooke, R.M., Pais de Barros, J.P., Wang, J.G., Aikawa, M., Masson, D., and Biddinger, S.B. (2016). Insulin dissociates the effects of liver X receptor on lipogenesis, endoplasmic reticulum stress, and inflammation. J. Biol. Chem. *291*, 1115–1122.

Takashima, M., Ogawa, W., Hayashi, K., Inoue, H., Kinoshita, S., Okamoto, Y., Sakaue, H., Wataoka, Y., Emi, A., Senga, Y., et al. (2010). Role of KLF15 in regulation of hepatic gluconeogenesis and metformin action. Diabetes *59*, 1608–1615.

Takeuchi, Y., Yahagi, N., Nakagawa, Y., Matsuzaka, T., Shimizu, R., Sekiya, M., Iizuka, Y., Ohashi, K., Gotoda, T., Yamamoto, M., et al. (2007). In vivo promoter analysis on refeeding response of hepatic sterol regulatory element-binding protein-1c expression. Biochem. Biophys. Res. Commun. *363*, 329–335.

Takeuchi, Y., Yahagi, N., Izumida, Y., Nishi, M., Kubota, M., Teraoka, Y., Yamamoto, T., Matsuzaka, T., Nakagawa, Y., Sekiya, M., et al. (2010). Polyunsaturated fatty acids selectively suppress sterol regulatory element-binding protein-1 through proteolytic processing and autoloop regulatory circuit. J. Biol. Chem. *285*, 11681–11691.

Teshigawara, K., Ogawa, W., Mori, T., Matsuki, Y., Watanabe, E., Hiramatsu, R., Inoue, H., Miyake, K., Sakaue, H., and Kasuga, M. (2005). Role of Krüppel-like factor 15 in PEPCK gene expression in the liver. Biochem. Biophys. Res. Commun. *327*, 920–926.

Uchida, S., Tanaka, Y., Ito, H., Saitoh-Ohara, F., Inazawa, J., Yokoyama, K.K., Sasaki, S., and Marumo, F. (2000). Transcriptional regulation of the CLC-K1 promoter by myc-associated zinc finger protein and kidney-enriched Krüppel-like factor, a novel zinc finger repressor. Mol. Cell. Biol. *20*, 7319–7331.

Urlinger, S., Baron, U., Thellmann, M., Hasan, M.T., Bujard, H., and Hillen, W. (2000). Exploring the sequence space for tetracycline-dependent transcriptional activators: novel mutations yield expanded range and sensitivity. Proc. Natl. Acad. Sci. USA *97*, 7963–7968.

Yahagi, N., Shimano, H., Hasty, A.H., Amemiya-Kudo, M., Okazaki, H., Tamura, Y., Iizuka, Y., Shionoiri, F., Ohashi, K., Osuga, J., et al. (1999). A crucial role of sterol regulatory element-binding protein-1 in the regulation of lipogenic gene expression by polyunsaturated fatty acids. J. Biol. Chem. *274*, 35840–35844.

Yahagi, N., Shimano, H., Hasty, A.H., Matsuzaka, T., Ide, T., Yoshikawa, T., Amemiya-Kudo, M., Tomita, S., Okazaki, H., Tamura, Y., et al. (2002). Absence of sterol regulatory element-binding protein-1 (SREBP-1) ameliorates fatty livers but not obesity or insulin resistance in Lep(ob)/Lep(ob) mice. J. Biol. Chem. *277*, 19353–19357.

Yahagi, N., Shimano, H., Matsuzaka, T., Najima, Y., Sekiya, M., Nakagawa, Y., Ide, T., Tomita, S., Okazaki, H., Tamura, Y., et al. (2003). p53 Activation in adipocytes of obese mice. J. Biol. Chem. *278*, 25395–25400.

Yahagi, N., Shimano, H., Matsuzaka, T., Sekiya, M., Najima, Y., Okazaki, S., Okazaki, H., Tamura, Y., Iizuka, Y., Inoue, N., et al. (2004). p53 involvement in the pathogenesis of fatty liver disease. J. Biol. Chem. *279*, 20571–20575.

Yamamoto, T., Shimano, H., Inoue, N., Nakagawa, Y., Matsuzaka, T., Takahashi, A., Yahagi, N., Sone, H., Suzuki, H., Toyoshima, H., and Yamada, N. (2007). Protein kinase A suppresses sterol regulatory element-binding protein-1C expression via phosphorylation of liver X receptor in the liver. J. Biol. Chem. *282*, 11687–11695.

Yin, L., Zhang, Y., and Hillgartner, F.B. (2002). Sterol regulatory element-binding protein-1 interacts with the nuclear thyroid hormone receptor to enhance acetyl-CoA carboxylase-α transcription in hepatocytes. J. Biol. Chem. *277*, 19554–19565.

Yoshikawa, T., Shimano, H., Amemiya-Kudo, M., Yahagi, N., Hasty, A.H., Matsuzaka, T., Okazaki, H., Tamura, Y., Iizuka, Y., Ohashi, K., et al. (2001). Identification of liver X receptor-retinoid X receptor as an activator of the sterol regulatory element-binding protein 1c gene promoter. Mol. Cell. Biol. *21*, 2991–3000.